Synthesis and structural analysis of sugar amino acid foldamers

Theses of Ph.D. dissertation

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Budapest, 2016
1. Introduction

The sugar amino acids (SAA) could include the features of carbohydrates and amino acids. The 5-, 6-membered furanoide and pyranoid rings in the peptide chain might be excellent peptide and protein spines because of their rigidity compared to the acyclic derivatives. These molecules can form secondary structures of proteins. In the light of these advantages, nowadays these molecules are in the centre of many scientific researches (e.g. to build into foldamers) [1].

The Exendin-4 (39 amino acids) as a peptide-type medicine for the treatment of diabetes-II and its modified derivatives are in the central researches of the MTA-ELU Protein Modeling group and the ELU Structural Biology and Chemistry Laboratory. One of the aim is to investigate structure, aggregation and folding processes of the modified Exendin-4 molecule and the Tc5b miniprotein and their derivatives [2-6].

To be based on this modern peptide medicine it is possible to design peptide models with shorter chain and to investigate their global structures. Resistance against enzymes could increase with β-sugar amino acids (β-SAA). The β-SAA as a biocompatible, polyfunctional and hydrophilic agent could build into the Exendin-4 molecule. Further significance of the oligomers of β-furanoid amino acids to contain tetrahydrofuran ring which is part of many biological active medicine molecules [7-9]. Various heterooligomers are created by combination β-SAA with α-amino acids.

On the score of the 1,2-O-isopropylidene-3-amino-3-deoxy-xylofuranuronic acid (8a) and its ribo epimer (8b) as β-furanoid sugar amino acids (βFSAA) Gruner and co-workers determined [8, 10] that 14-helix structure formed in foldamer peptides including the xylo (3,4-cis) (8a) configuration monomers. It might be excellent to the mimicry of α-helix [8, 10-11]. The 5-, or 6-membered ring sugar amino acids as building blocks could be suitable to build carbopeptides.
2. Aims

1. New, high-scale and profitable preparation of 1,2-O-isopropylidene-3-amino-3-deoxy-\(\alpha\)-D-xylofuranuronic acid (H-XylAFU(ip)-OH) (8a) monomer from D-glucose on various pathways and its global elucidation.

2. Preparation and characterization of the (8a) N-, and C-protected derivatives.

3. Preparation and structural analysis of two diamide-derivatives C\(^3\)-(NHCOMe) and C\(^4\)-(CONHMe)/ C\(^4\)-(CONMe\(_2\)) as model SAA part in peptide bond.

4. Investigation and optimization of Staudinger reactions on the 1,2-O-isopropylidene-3-azido-3-deoxy-\(\alpha\)-D-xylofuranuronic acid (7) as well as the N- and C-protected azido-derivatives H-XylAFU(ip)-OH (8a).

5. Peptide coupling from the N- and C-protected derivatives of (8a) to various homooligomers in solution and on solid phase.

6. Building up the (7) molecule (X) between two diglycyl dipeptide, thus preparation of a hydrophilic GGXGG sequence model \(\beta\)-peptide on solid phase with Staudinger reaction and Fmoc/O\textsuperscript{t}Bu peptide coupling method.

7. Preparation, purifying and FTIR-spectroscopic analysis of the Tc5b miniprotein with 5 and 10 amino acids elongated derivatives (H5 és H10) to investigate their aggregation.

8. Multi-building up the (7) monomer (X) to the variable part of Exendin-4 peptide. (Scheme 4.) thus preparation of the \(\beta\)-Exendin-4 with increased resistance on solid phase with Staudinger reaction and Fmoc/O\textsuperscript{t}Bu method.

9. Global structural analysis of the prepared SAA derivatives, their homooligomers, the GGXGG \(\beta\)-peptide, and the \(\beta\)-Exendin-4 by spectroscopic methods.

10. Supporting of the experimental observations with theoretical, quantum chemical calculations.
3. Materials and methods

The H-XylAFU(ip)-OH (8a) derivatives and their homooligomers were prepared by conventional carbohydrate and peptide chemistry synthetic methods in solution. The products were mainly purified on Kieselgel 60 silica gel (0.040-0.063 mm; Merck) by column chromatography. The reactions were followed by TLC Kieselgel 60 F254 (E. Merck). The developments were occurred under UV-light (254 nm) and in sulphuric acid solution (5%). The melting points of the solid products were measured by Boetius microscope type apparatus. The optical rotations were determined by Carl Zeiss Jena Polamat A type polarimeter.

The β-peptide derivatives were built on various solid phase (2-Cl-Trt, RinkAmid MBHA and Wang resin) by Fmoc/OtBu method with different coupling protocols. Purity of several product was controlled by RP-HPLC on Phenomenex Jupiter C18 (21.2x250 mm) column in water/acetonitrile containing 0.1% TFA (A eluent), as well as 80% acetonitril/water mixture containing 0.1% TFA (B eluent) solvent, with gradient elution (0-40% B eluent through 80 minutes), 3 ml/min flow velocity and UV detection (on 220 and 280 nm wavelength).

After the HPLC purifying the product peptides were lyophilized and analysed by Bruker Esquire 3000+ tandem kvadrupol detection electrospray mass spectrometer.

The FTIR spectras of H0, H5 and H10 were measured by Bruker-Equinox-55 spectrometer with MCT detector, in BaF2 cuvette with 0.05 mm fix path lenght, between 4000-750 cm⁻¹, 4 cm⁻¹ resolution in D2O. Every spectra was corrected by the solvent absorption. The spectras of H5 were measured with 0.36-7.2 mM concentration, on pH, at 25 °C temperature. The pH-dependent measurements were carried out in (pH 4.0; pH 5.4 and pH 7.0) 1.0-2.5 mg/ml (0.36-0.72 mM) concentration a. In the case of H10 the curves were determined between 1.0-2.5 mg/ml (0.3-0.6 mM) on pH 3 and pH 7 at 25 °C temperature. The spectra resolutions were carried out by OPUS 6.0 software with Curve Fit method.

Supporting of the experimental experiences were carried out by quantum chemical calculations: The conformational search was the 10000 steps Monte Carlo Multiple Minimum (MCMM) method. In every Monte Carlo step the iminophosphoranes torsion angles were changed random between 0-180°. The energy of the further structures was minimized by the Pollack-Ribriere conjugated gradient method. The structures were kept between 0-21 kJ/mol from the global minimum. OPLS field [135 a, b] was aplied for the calculations and the solvation effect was modelling by (MeOH) GB/SA algorithm. The most representative
structures were chosen to the further optimization. The structure and energy optimization were carried out by DFT method (M06-2x/6-311++G**, PBF solvation algorithm in methanol) and Schrödinger Jaguar [135c] program. On the other hand some calculations of iminophosphoranes were carried out by Gaussian 09 program at B3LYP/6-31+G(d) theory level with IEFPCM solvent model in vacuo and in water. The natural bond orbital analysis was performed by NBO 5.9 program.

The identification of the products and intermediates are carried out by Bruker Avance 250 NMR spectrometer at room temperature (301K) in deuterated solvents (CDCl₃, D₂O, CD₃OD, DMSO-d₆, MeCN-d₃). The 2D homo- and heteronuclear NMR spectroscopic measurements of β-peptides were occured by Bruker Avance-III 700 spectrometer.

4. Results and summary

A high-scale and profitable synthetic pathway of the 1,2-\(O\)-isopropylidene-3-amino-3-deoxy-\(\alpha\)-D-xylofuranuronic acid (8a) (H-XylAFU(ip)-OH) was elaborated (Scheme 1.).

![Scheme 1. H-XylAFU(ip)-OH-8a synthetic pathway](image)

A new synthetic method was developed for the preparation of new diamide-derivatives (21 és 22) (Scheme 2.). Methylamine and dimethylamine were coupled via anhydride to the (7) monomer C\(^3\)(-COOH) group by peptide coupling method in solution. The C\(^3\)(-N\(_3\)) group was reduced by H-Cube mini flow reactor with H\(_2\) gas on 10% Pd/C catalyst in MeCN solvent.
The formation of C\(^3\)(-NHAc) function was carried out with Ac\(_2\)O/Py mixture. The chiroptical spectroscopic measurements of (21) and (22) molecules showed that the monomer lead the whole β-peptide into β-, γ-turn and PPII structures.

![Scheme 2](image)

Scheme 2. The N-methyl- (21) (H-XylAFU(ip)-NHMe) and the N,N-dimethyl-3-acetylamido-3-deoxy-1,2-O-isopropylidene-α-D-xylofuranuronamide (22) (H-XylAFU(ip)-NMe\(_2\)) model compounds.

The direct formation of the azido function was successfully occured by Staudinger reaction in mild reaction conditions with PBu\(_3\) reagent in MeOH:THF 1:1 mixture in solution and on solid phase. The iminophosphorane hydrolysis to free amine compound were carried out in MeOH:THF 1:1 mixture containing TEA/DIEA. Thus possibilities of scale up preparation and automation of the reactions of β-peptides were opened with application of easily removable phosphines.

Furthermore the profound investigation of Staudinger reactions of 3-azido-3-deoxy-furanose derivatives were successful. Due to this effort, verification of the unexpected stability of the N-methyl-3-deoxy-3-triphenylphosphinimino-1,2-O-isopropylidene-α-D-xylofuranuronamide (9B) was effecient supporting with quantum chemical and X-ray diffraction methods. The reason of the unexpected stability was the H-bond between the oxygen of the furanose ring and the C-4 carboxamide NH-proton. In consequence the C-4 carbonyl group was oriented towards the phosphinimine P-atom, thus blocking the attaction of the nucleophile therefore the hydrolysis to free amine compound was not occured.

The further part of the PhD research the N\(_3\)-XylAFU(ip)-OH (7) precursor (=X) was built up successfully into α-peptides with transformation of azido-function by Staudinger reaction, it was optimized on solid phase and automation of this method was prepared during building of GGXGG sequence model peptides. The increased hydrophilicity and the solubility of the β-polypeptide in water were reached successfully with application of βFSAA monomer.

Furthermore the wide-range structure analyses of the model precursor molecules, the homodimers and the β-heteropentamer (GGXGG) containing H-XylAFU(ip)-OH were carried out successfully. As a result of this elucidation that the βFSAA was a producing of chiral induction agent. In addition the monomer was led the β-polipeptide to helix structure.
Thus the foldamers containing H-XylAFU(ip)-OH are excellent biopolimers for the mimicry of α-helix.

The long-range aim of the research is to build up the β-Exendin peptide. I carried out experiments to build up the N₃-XylAFU(ip)-OH (7) precursor (=X) into the variable part of the Exendin-4 (Scheme 3.) molecule with the synthetic pathway of GGXGG model peptides:

<table>
<thead>
<tr>
<th>β-Ex-4:</th>
<th>39</th>
</tr>
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<tbody>
<tr>
<td>HGEGTFTSDL</td>
<td>SKQMEEE AV RLYIQWLGKGPSSGRPPPS</td>
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**Scheme 3.** Above: The Exendin-4 peptide medicine for the treatment of diabetes II. Below: Building up the (7) SAA precursor into the variable part of the Exendin-4.

Preparation of the complex sequence fragment containing βFSAA was carried out successfully by step by step solid phase peptide synthesis (SPPS) with Fmoc/O'Bu method.

In one hand the results was not displayed in the thesis but these pre-experiments were opened a new pathway for the research group to build up the β-Exendin-4 molecule with short half-life period to increase the resistance against enzymes.

On the other hand the aggregation process of the Tc5b derivatives elongated with 5 and 10 amino acids (H5 és H10) (Scheme 4.) was investigated successfully by optical spectroscopic methods.

**Scheme 4.** The structure of Exendin-4 (Ex-4), its variable part (black) as well as the sequence of the Ex-4, the Tc5b, the H5 and the H10.

The aggregation was exclusively occurred in strenuous circumstances due to external inductions. These results predict for the future research that the homooligomers containing βFSAA building block could significantly improve the aggregation features.
5. References


[6] a) Rogne, P; Ozdowy, P; Richter, C; Saxena, K; Schwalbe, H; Kuhn, LT; *PLoS ONE*, 2012, 7, e41301 b) Rovó, P; Stráner, P; Láng, A; Bartha, I; Huszár, K; Nyitray, L; Perczel, A; *Chem.Eur.J.*, 2013, 19, 2628-2640 c) Rovó, P; Farkas, V; Stráner, P; Tóth, KG; Perczel, A; 2013, in preparation.


[9] b) Sharma, GVM; Reddy, PS; Chatterjee, D; Kunwar, AC; *J. Org. Chem.*, 2011, 76, 1562–1571;


List of own publications

1. Farkas, V; Csordás, B; Hegyi, O; Tóth, KG; Perczel, A; Foldamer Stability Coupled to Aggregation Propensity of Elongated Trp-Cage Miniproteins; 

2. Csordás, B; Nagy, A; Karancsi, MD; Harmat, V; Zsoldos-Mády, V; Leveles, I; Farkas, V; Pintér, I; Perczel, A; 
Origin of problems related to Staudinger reduction in carbopeptide syntheses; 
*Amino Acids*, **2016**, Submission ID: AMAC-D-16-00176R1

3. Nagy, A; Csordás, B; Zsoldos-Mády, V; Pintér, I; Farkas, V; Perczel, A; C-3 epimers of sugar amino acids as foldameric building blocks: improved synthesis, useful derivatives, coupling strategies; 
*Amino Acids*, **2016**, Submission ID: AMAC-D-16-00294

Conference presentations

1. Barbara Csordás, Viktor Farkas, Gábor Tóth, András Perczel: 
Among the concentrations: between the IR, ECD and VCD 
*MTA Peptide Chemistry Committee Congress, Balatonszemes, 30 May – 1 June 2012*

2. Barbara Csordás, Viktor Farkas, Gábor Tóth, András Perczel: 
Synthesis and structural analysis of foldamers 
*XVIII. National Chemist Conference, Félixfürdő, 22-25 Nov 2012*

3. Barbara Csordás, Viktor Farkas, András Perczel: 
Synthesis and structural analysis of foldamers 
*MTA Peptide Chemistry Committee Congress, Balatonszemes, 29-31 May 2013*

4. Barbara Csordás, Viktor Farkas, András Perczel 
Synthesis of sugar amino acid foldamer precursors 
*MTA Coordination Chemist and Peptide Chemistry Committee Congress, Budapest, ELU, 28 Oct 2013*

5. Barbara Csordás, Viktor Farkas, András Perczel 
Synthesis of sugar amino acid foldamer precursors 
*XXXVI. Chemistry Presentation Days, Szeged, 28-30 Oct 2013*
6. **Barbara Csordás**, Viktor Farkas, András Perczel

Synthesis of sugar amino acid foldamer precursors

*XIX. National Chemistry Conference, doctoral plenum, Nagybánya, Romania, 21-24 Nov 2013*

7. **Barbara Csordás**, Viktor Farkas, András Perczel

Synthesis and structural analysis of sugar amino acid foldamer precursors

*MTA Peptide Chemistry Committee Congress, Balatonszemes, 28-30 May 2014*

**Foreign language conference presentation:**

**Barbara Csordás**, Viktor Farkas, András Perczel

Synthesis and structural analysis of new sugar amino acid foldamers

*MTA Carbohydrate, Nucleic Acid and Antibiotics Committee Congress, Mátraháza, 21-23 May 2014*

**Thesis pre-defence:**

**Barbara Csordás**

Synthesis and structural analysis of sugar amino acid foldamers

*MTA Carbohydrate, Nucleic Acid and Antibiotics Committee Congress, Mátraháza, 27-29 May 2015*

**Posters:**

1. **Barbara Csordás**, Viktor Farkas, András Perczel:

Synthesis of precursors of sugar amino acid foldamers

*5th European Conference on Chemistry for Life Sciences (5th ECCLS), Barcelona, 10-12 June 2013*

2. **Barbara Csordás**, Viktor Farkas, András Perczel:

Synthesis of precursors of sugar amino acid foldamers

*6th Central Europe Conference – Chemistry towards Biology, Trieste, 10-13 Sept 2013*